H3K9me2 attracts PGC7 in the zygote to prevent Tet3-mediated oxidation of 5-methylcytosine

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Epigenetic reprogramming of the parental genome occurs in fertilized oocytes and involves oxidation of 5-methylcytosines (5-mC) to 5-hydroxymethylcytosines (5-hmC) in the paternal pronucleus. Recent work has shown that the maternal genome is protected from this remodeling step by an interaction between a modified histone, H3K9me2, and the oocyte-derived factor, PGC7, to prevent oxidation of the maternal DNA by the Tet3 5-methylcytosine oxidase.

Soon after mammalian fertilization, a global epigenetic remodeling process takes place, leading to a totipotent epigenetic state. Global removal of the gametic DNA methylation occurs very fast in the paternal genome, as visualized by a rapid loss of immunostaining with 5-mC antibody in the paternal pronucleus during zygote maturation (Mayer et al., 2000). The mechanism of rapid ‘demethylation’ of the paternal pronucleus involves global oxidation of 5-mC to 5-hmC during the pronuclear stages (Gu et al., 2011; Iqbal et al., 2011; Wossidlo et al., 2011) and is catalyzed by Tet3 methylcytosine dioxygenase (Gu et al., 2011; Wossidlo et al., 2011) (Figure 1A). PGC7 (also known as Dppa3 or Stella) protein, however, protects the maternal pronucleus from the fast global ‘demethylation’ (Nakamura et al., 2007), by inhibiting 5-mC to 5-hmC conversion (Wossidlo et al., 2011). Both pronuclei lose 5-mC signals and accumulate 5-hmC signals in zygotes derived from homozygous mutant (PGC7−/−) mothers (Figure 1).

Nakamura et al. (2012) now asked further questions. Why does PGC7 specifically protect the maternal pronucleus from 5-mC to 5-hmC conversion, if it is present in both pronuclei, and how does PGC7 prevent oxidation of 5-mC by Tet3? The authors confirmed that PGC7 is present in both pronuclei, but also revealed that it binds with higher affinity in the maternal pronucleus. This was visualized by inverting the permeabilization and fixation steps in the staining procedure. Why is PGC7 more strongly attached to the maternal pronucleus? The authors hypothesized that the difference lies in chromatin composition. Indeed, H3K9me2 associates with methylated DNA in the maternal pronucleus (Santos et al., 2005). In support of the hypothesis, Nakamura et al. (2012)
found that PGC7 specifically binds to H3K9me2 peptides via its N-terminus. Also, PGC7 more strongly associates with H3K9me2-rich nucleosomes than with H3K9me2-depleted nucleosomes obtained from ES cells that lack the histone H3K methyltransferase G9a. When H3K9me2 levels were reduced in the zygote by ectopic expression of Jhdm2a (an H3K9me2 demethylase), PGC7 was not bound strongly to the maternal pronucleus, resulting in 5-mC loss and 5-hmC accumulation in the maternal pronucleus (Figure 1B). Taken together, the authors concluded that PGC7 is strongly associated with the H3K9me2-rich maternal pronucleus, and this association is required for protecting from 5-mC to 5-hmC conversion. How does PGC7 protect the maternal pronucleus from Tet3-mediated oxidation? The authors went on to show that PGC7 is required for excluding Tet3 from the maternal pronucleus. Overexpressed Tet3 was strongly associated only with the paternal pronucleus in normal zygotes. In PGC7-depleted zygotes, however, strong binding of Tet3 occurred in both pronuclei. The same was observed when PGC7 nuclear import was inhibited or when H3K9me2 was removed from the zygote by Jhdm2a expression. How does PGC7 exclude Tet3 from the chromatin of the maternal pronucleus? Using chromatin salt extraction in ES cells overexpressing the different proteins, the authors showed that PGC7 via its C-terminus reduces Tet3 affinity to chromatin. They also showed that in ES cell chromatin extracts, PGC7 protects linker DNA from micrococcal nuclease digestion, again depending on PGC7’s binding to H3K9me2-enriched nucleosomes. The authors concluded that PGC7 inhibits Tet3-mediated oxidation of 5-mC in the maternal pronucleus by altering chromatin configuration.

PGC7 not only protects the maternal pronucleus from global demethylation, but also protects certain genomic imprints during the wave of zygotic demethylation in the paternal pronucleus and, even in the paternal pronucleus (Nakamura et al., 2007). Genomic imprints are parental allele-specific DNA methylation marks at the differentially methylated regions (DMRs) of imprinted genes/domains. Gametic DMR methylation is inherited from the gametes, protected during the global epigenetic remodeling events in the embryo and later maintained in the soma. PGC7 specifically protects the paternally methylated H19-igf2 and Rasgrf1 DMRs but not the IG-DMR1 in the paternal pronucleus. It is not known whether the PGC7-protected DMRs are associated with H3K9me2 in the respective pronuclei, but Nakamura et al. (2012) show that this is very likely the case, because the zygote receives the H19-igf2 and Rasgrf1 DMRs but not the IG-DMR1 from the sperm in association with H3K9me2. The authors then conclude that PGC7 protects certain gametic imprints from 5-mC oxidation, because H3K9me2 allows tight PGC7 association at these loci in the paternal pronucleus, which in turn can reduce Tet3 affinity to these regions.

Two additional proteins have been implicated in protecting genomic imprints: Zfp57, a KRAB domain 2n finger transcription factor and Trim28 (KAP1), both being part of an epigenetic modifier complex. The three proteins have overlapping and also distinct patterns of protection at DMRs. PGC7 specifically protects the paternally methylated H19-igf2 and Rasgrf1 DMRs and paternally methylated Peg1, Peg3, and Peg10 DMRs. PGC7 is not required for protecting the Snrpn and Peg5 maternal DMRs and the IG-DMR1 (Meg3) paternal DMR. Zfp57 is required for protecting the IG-DMR1 but not the H19-igf2 paternal DMRs and the Peg1, Peg3, Peg5, and Snrpn and maternal DMRs during preimplantation (Li et al., 2008). In addition, Zfp57 is required to protect the Rasgrf1, IG-DMR and H19-igf2 paternal, and the Gnas/Nespa, KvDMR1, Peg3, and Snrpn maternal DMRs in ES cells (Quenneville et al., 2011; Zuo et al., 2012). Trim28/KAP1 is required in a stochastic fashion for protecting the H19-igf2 and IG-DMR1 paternal DMRs and Peg3 and Snrpn maternal DMRs (Messerschmidt et al., 2012). The three proteins exhibit differences also in protecting methylation of repeat elements. PGC7 protects IAPs but not Line1 repeats from demethylation. However, Trim28 does not protect IAPs in ES cells (Quenneville et al., 2011) and Zfp57 does not protect either IAPs or Line1s in the embryo and only slightly protects IAPs in ES cells (Li et al., 2008; Zuo et al., 2012).

PGC7, Zfp57, and Trim28 are all expressed in the oocyte and during cleavage, and all three are important for development. PGC7 and Trim28 are maternal effect genes: lack of these proteins in the oocyte results in highly penetrant lethality (Payer et al., 2003; Messerschmidt et al., 2012). PGC7 maternal mutant homozygous PGC7m−/− embryos die before implantation. The zygotic PGC7 cannot fully rescue preimplantation development in maternal mutant heterozygous PGC7m−/+ offspring (Payer et al., 2003). Trim28m−/+ embryos die at variable times starting at postimplantation but always before birth (Messerschmidt et al., 2012). Zfp57 mutation results in maternal-zygotic lethality (Li et al., 2008): Zfp57m−/+ offspring die around mid-gestation, but Zfp57 zygotic transcript can rescue the development in Zfp57m−/+ offspring. Interestingly, PGC7−/− offspring is viable, but half of Zfp57−/− offspring dies at variable time peri- or neonatally. This means that maternal PGC7 is sufficient for development to term, but Zfp57 zygotic expression is also important. The death of the maternal or maternal-zygotic respective mutants is most likely caused by the failure of protecting the DNA from demethylation during early embryonic development and the consequent aberrant expression of development-regulating genes, including imprinted genes, during gestation. PGC7’s maternal effect results in much earlier death than that of Trim28, likely because PGC7 protects the entire maternal pronucleus (Nakamura et al., 2007), whereas Trim28m−/− zygotes are not deficient in the protection of the maternal pronucleus (Messerschmidt et al., 2012). It is not known whether Zfp57 has any role in protecting the maternal pronucleus from global demethylation, but this is not likely based on the fact that zygotic Zfp57 can rescue the maternal lethality phenotype.

Whereas the association of PGC7 with nucleosomes depends on H3K9me2 (Nakamura et al., 2012), it is not known whether H3K9me2 affects Zfp57 and Trim28 affinity to chromatin. PGC7 binds to DNA in a CpG methylation-insensitive fashion (Nakamura et al., 2007), whereas Zfp57 and Trim28 are associated with methylated DNA (Quenneville et al., 2011). Association of Trim28 at DMRs depends on DNA methylation: it is absent in Dnmt triple knockout ES cells. Trim28 enrichment at DMRs also depends on Zfp57. These two proteins are bound in vivo in the methylated parental alleles.
of DMRs in ES cells (Quenneville et al., 2011) and (at least Trim28 at the H19-Igf2 DMR) in mid-gestation stage embryos (Messerschmidt et al., 2012). Zfp57-Trim28-Setdb1 triple occupied ChIP-sequencing peaks in ES cells defined a consensus hexanucleotide sequence, TGC\(^n\)CGC where the CpG site is methylated (Quenneville et al., 2011). This consensus is present in each known DMR.

Whereas PGC7 protects from demethylation by inhibiting Tet3-mediated oxidation of 5-mC, Zfp57, and Trim28 appear to protect specific sequences from demethylation by arresting repressing epigenetic modifiers to the target sequences and by facilitating heterochromatinization and DNA remethylation (Quenneville et al., 2011; Zuo et al., 2012). Zfp57 and Trim28 coexist with a repressive histone mark, H3K9me3 together with the H3K9me3 histone methyltransferase Setdb1 at the Snrpn, Peg3 DMRs and at the IG-DMR in 12.5 dpc embryos (Messerschmidt et al., 2012). This complex includes HP1 and Uhrf1 (NP95) at the methylated alleles of DMRs in ES cells (Quenneville et al., 2011). Trim28 binding depends on DNA methylation and in turn H3K9me3 enrichment at DMRs depends on Trim28 (at least) in ES cells (Quenneville et al., 2011). This may underlie the fact that repressive chromatin (H3K9me3-rich and H3K4me2-poor, for example) in the maternal allele at several maternal DMRs depends on DNA methylation, as shown in Dnmt3L\(^{-/-}\) embryos (Henckel et al., 2009). Trim28 binds to Dnmt proteins, Dnmt1, Dnmt3a, Dnmt3b, and Dnmt3L, thus recruiting Dnmts to methylated (or partially demethylated) DNA, bringing the remethylation events to a full circle (Quenneville et al., 2011; Zuo et al., 2012). It is interesting that Uhrf1 can bind not only methylated but also hydroxymethylated DNA (Frauer et al., 2011).

It is tempting to speculate that Uhrf1 may thus help reverse 5-hmC marks to 5-mC marks during DNA replication by bringing in Dnmts to the nascent strand.

The timing of methylation protection appears to be shifted between PGC7 and the other two proteins. The PGC7-mediated H3K9me2-dependent protection seems to be very specific to the very early event in the zygote's maternal pronucleus, likely because of the restricted expression pattern of Tet3 (Gu et al., 2011; Iqbal et al., 2011; Wossidlo et al., 2011). Global DNA methylation does not depend on H3K9me2 in ES cells any more, as G9a null ES cells that have greatly reduced H3K9me2 levels have normal 5-mC levels (Nakamura et al., 2012). Trim28 and Zfp57, however, exhibit their protective functions during cleavage stages (Messerschmidt et al., 2012) and likely function as late as in the blastocyst, because Zfp57\(^{-/-}\) and Trim28\(^{-/-}\) ES cells are deficient in imprint maintenance (Quenneville et al., 2011; Zuo et al., 2012).

Further studies will need to address the mechanistic details of how PGC7 deters Tet3 from chromatin. It will be also important to determine whether PGC7 binds to the methylated alleles of DMRs in early development. Other open questions are what determine H3K9me2 levels at paternal DMRs in sperm and when this mark is attained, before or after de novo methylation in prospermatogonia, or only at spermiogenesis. At least at the global level, H3K9me2 accumulates in prospermatogonia before global de novo DNA methylation takes place (Abe et al., 2011). Locus-specific examination will be necessary to find out if this occurs at paternal DMRs.

References


